IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: TAKAGAKI, Hidetsugu et al.
Application No.: 10/565,828
Filling Date: 01/25/2006
Group Art Unit: 1612 Examiner: SIMMONS, CHRIS E
Title: THERAPEUTIC AGENT FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND METHOD FOR TREATING CHRONIC OBSTRUCTIVE PULMONARY DISEASE USING THE SAME

DECLARATION PURSUANT TO 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Yasuo AOKI, residing in Yotsukaido-shi, Japan, declare and state that:

1. I graduated with a Ph.D. from Toho University in August, 2001. Since April 1985, I had been employed by Dainippon Ink and Chemicals, Inc., and at the time of the invention, I was engaged in research and development.

2. I am one of the inventors of the invention as claimed in the above-referenced application, and accordingly, I am familiar with the specification and claims which compose the application.

3. I am aware of the Office Action of January 31, 2008, issued on the above-referenced application, in which claims 15, 17-20, 22, 24, 26, 28 and 30-32 of the present invention were rejected under 35 U.S.C. 103(a) as being unpatentable over Kimura *et al.* (*Chem. Pharm. Bull.* (2001); 49(10): 1321-1325) in view of Postma *et al.* (Am J Respir Crit Care Med. (1998); 158(5 Pt 3): S187-92).

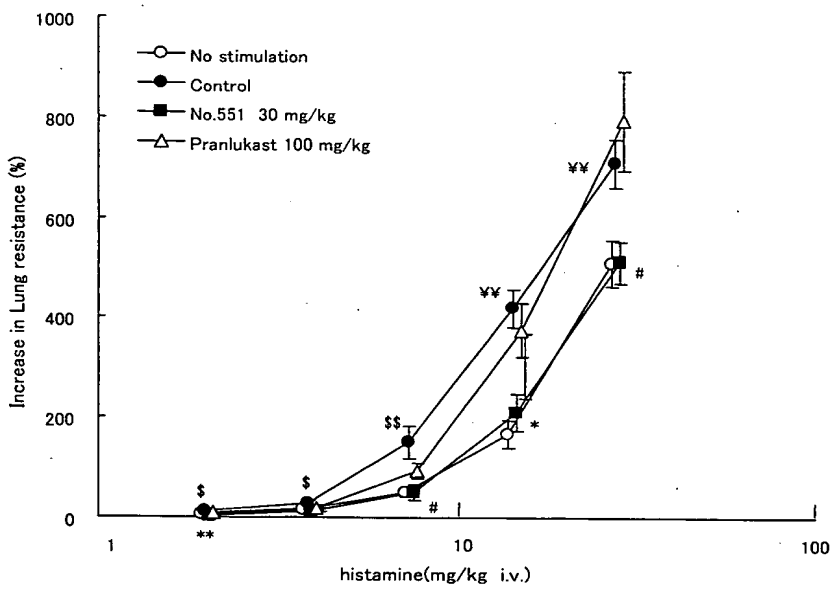
4. As is described in this Declaration, I conducted experiments for the purpose of demonstrating that unexpected effects can be obtained by the claimed subject-matter,

and the experimental procedures and obtained data are presented below.

Experiment 1

A commercially-available antiasthmatic drug "pranlukast" was studied on an airway hyper-responsiveness model induced by exposure of peroxynitrite in guinea pigs. 100 mg of the test drug was orally administered to a guinea pig in the same manner as Example 2 in the present specification to evaluate the effects of the drug on airway hyper-responsiveness in terms of lung resistance. The results are shown in FIG. 1 below. For comparison, compound No. 551, a "no stimulation group" and a control group are also shown in FIG. 1.

FIG. 1



Effects of Compound No. 551 of the present invention and Pranlukasut on the increases in lung resistance induced by peroxynitrite
N=12
$$: P<0.01 (No stimulation vs Control; Aspin-welchi t-test)
¥¥:P<0.01 (No stimulation vs Control; Student t-test)
#:P<0.05, ##:P<0.01 (Control vs No.551; Steel multiple test)
**:P<0.01 (Contro vs No.551; Dunnett's multiple test)

Experiment 2

Effects of compound No. 551 and a commercially-available drug "theophylline" generally used for treating asthma and COPD were evaluated on an emphysema model induced by a cigarette smoke solution in guinea pigs. Materials and procedures thereof are summarized below.

MATERIALS AND METHODS

· Animals

Male, 5-week-old, Hartley guinea pigs weighing 300-350 g were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in a temperature-controlled room at 19 to 25°C under a daily 12-hour light/dark cycle. All of the experimental procedures were approved by the Institutional Animal Care and Use Committee of Kobuchisawa Research Laboratories, Fuji Biomedix Co., Ltd. (Yamanashi, Japan).

· Reagents

LPS (Escherichia coli, serotype 055:B5) and Boc-L-alanine 4-nitrophenyl ester were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Theophylline was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hi-Lite cigarettes were purchased from Japan Tobacco Inc. (Tokyo, Japan).

By using an air pump to generate suction, CSS was prepared by bubbling a stream of the smoke into saline (40 mL•40 cigarettes-1). It took approximately 3 min to bubble the smoke from one cigarette into the saline. The absorbance of a 100-fold dilution of CSS with saline was 1.307 at a wavelength of 267 nm. This original solution was used for the intratracheal administrations.

· Study design

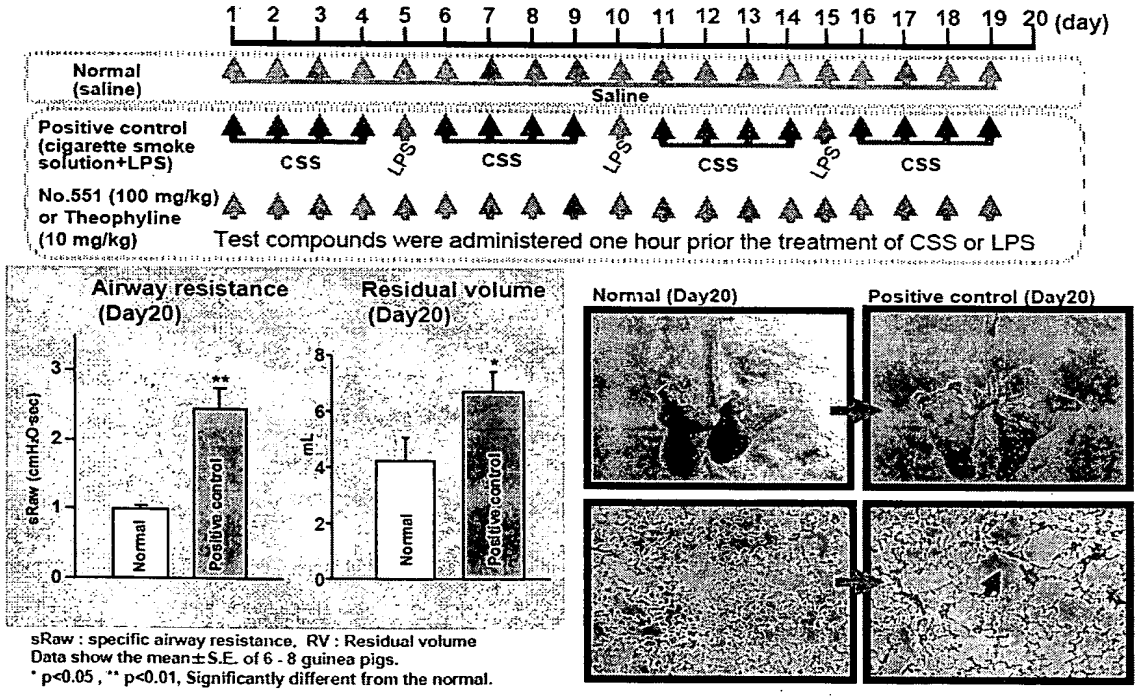
The protocol followed in this study is summarized in Fig. 2 below. CSS (200 μL•animal-1 per time) was intratracheally administered once a day on days 0-3 without the use of general anesthetics. Subsequently, a LPS solution (500 μg•mL-1, 200 μL•animal-1 per time) was intratracheally administered on day 4. These 4 CSS and 1

LPS administrations were regarded as being cycle 1, with cycles 2 and 3 conducted on days 5-9 and 10-14, respectively. CSS was then administered on days 15-18 (CSS+LPS group). Time-related changes in the specific airway resistance (sRaw) were measured on days 0, 4, 9, 14 and 19. On days 0, 4, 9 and 14, measurements of sRaw were performed prior to the administration of CSS or LPS. On day 19, residual volume (RV) was investigated. As a negative control, saline was administered intratracheally once a day on days 0-18 (normal group).

To evaluate the effect of compound 551 (100 mg•kg-1) and theophylline (10 mg•kg-1) were orally administered once a day 1 hour before the respective intratracheal instillations of CSS or LPS on days 0-18 (Theophylline group).

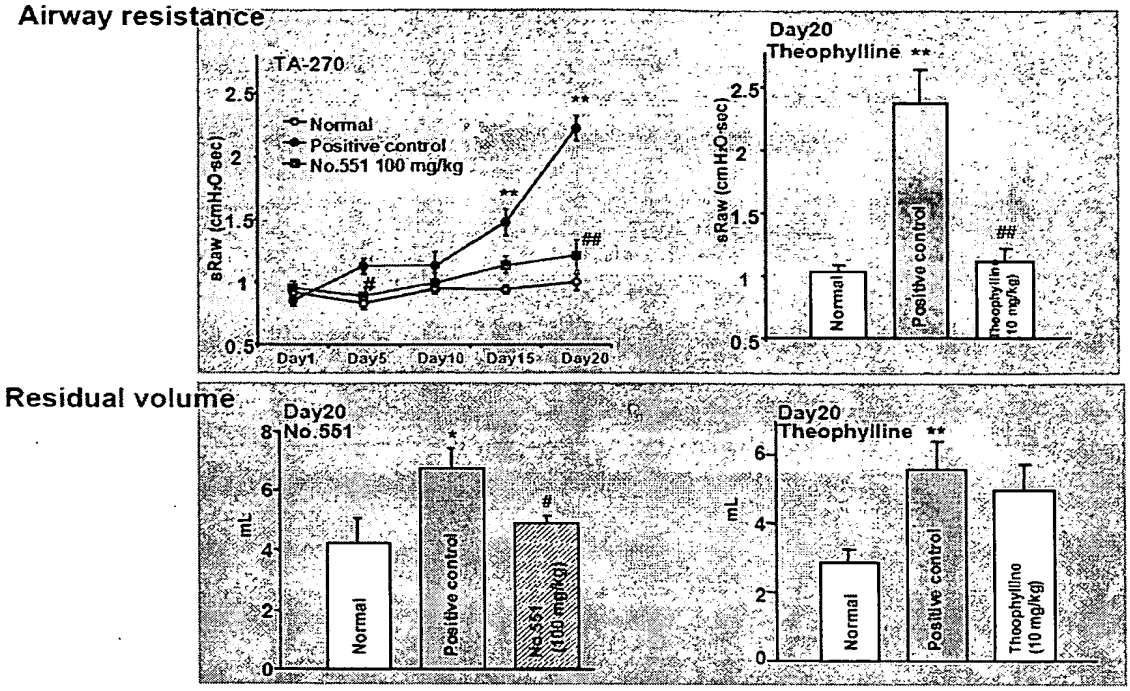
The results are shown in FIG. 3.

FIG. 2



sRaw : specific airway resistance, RV : Residual volume
Data show the mean±S.E. of 6 - 8 guinea pigs.
* p<0.05 , ** p<0.01, Significantly different from the normal.

FIG. 3



Data show the mean ± S.E. of 6 - 8 guinea pigs.
*** $p<0.05$, ** $p<0.01$, Significantly different from the normal.**
 # $p<0.05$, ## $p<0.01$; Significantly different from the positive control.

Experiment 3

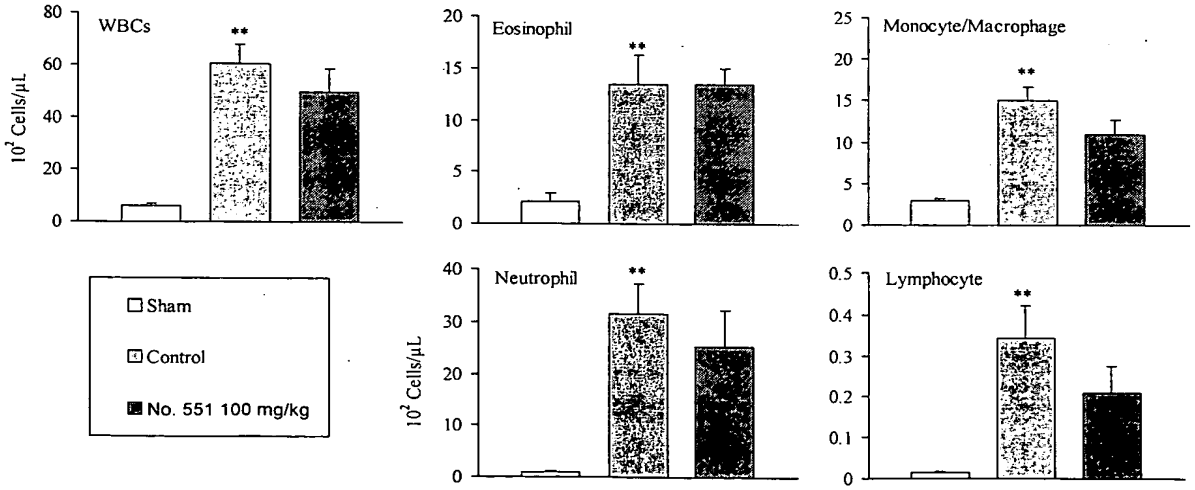
The effects of compound No. 551 on infiltration of inflammatory cells into the bronchoalveolar cavity were further evaluated on the above-described emphysema model induced by a cigarette smoke solution in a guinea pig, based on the following cell-counting method.

Method for counting of inflammatory cells

Immediately after assessment of the pulmonary function, lungs were lavaged via the tracheal tube with saline (5 mL x 2). The recovered lavage fluid was centrifuged at 120 x g for 5 min at 4°C. The cell pellet was suspended with a defined volume (200-800 μL·sample-1) of saline. The total leukocyte count in the lavage fluid was determined by staining with Turk's solution. For the differential cell counts, BAL cells were centrifuged onto a glass slide by using Cytospin followed by staining with Diff-Quik solution. A Minimum of 500 cells were counted under a microscope and, based on their morphological criteria, classified as being macrophages, lymphocytes, neutrophils or eosinophils.

The results are shown in FIG. 4.

FIG. 4



Effect of compound No. 551 on filtration of inflammatory cells into the bronchoalveolar cavity in emphysema model of guinea pig.
Each value represents the mean with standard error.
* $p<0.05$, ** $p<0.01$; Significantly different from the sham group.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 16, 2008

Yasuo Aoki

Yasuo AOKI